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Toxicological assessment of kretek cigarettes
Part 2: Kretek and American-blended cigarettes, smoke chemistry and *in vitro* toxicity

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ABSTRACT

Two commercial kretek cigarettes typical for the Indonesian market and a reference kretek cigarette were compared to the American-blended reference cigarette 2R4F by smoke chemistry characterization and *in vitro* cytotoxicity and mutagenicity assessments. Despite the widely diverse designs and deliveries of the selected kretek cigarettes, their smoke composition and *in vitro* toxicity data present a consistent pattern when data were normalized to total particulate matter (TPM) deliveries. This confirms the applicability of the studies' conclusions to a wide range of kretek cigarette products. After normalization to TPM delivery, nicotine smoke yields of kretek cigarettes were 29–46% lower than that of the 2R4F. The yields of other nitrogenous compounds were also much lower, less than would be expected from the mere substitution of one third of the tobacco filler by clove material. Yields of light molecular weight pyrolytic compounds, notably aldehydes and hydrocarbons, were reduced, while yields of polycyclic aromatic hydrocarbons were unchanged and phenol yield was increased. The normalized *in vitro* toxicity was lowered accordingly, reflecting the yield reductions in gas-phase cytotoxic compounds and some particulate-phase mutagenic compounds. These results do not support a higher toxicity of the smoke of kretek cigarettes compared to American-blended cigarettes.

1. Introduction

This publication is part of a series summarizing the *in vitro* and *in vivo* toxicological assessment of kretek cigarettes. Smoke composition and biological activity of mainstream smoke (MS) from marketed and experimental kretek cigarettes were evaluated on a comparative basis in smoke chemistry analyses, and *in vitro* and *in vivo* toxicity studies. The studies were designed to cover three main topics: (1) characterization of kreteks and comparison relative to American-blended cigarettes, (2) impact of blend type and cloves, and (3) impact of ingredients used in kretek cigarettes. Further in depth information of this assessment is described in the lead publication (Roemer et al., 2014c).

As part of the characterization of kretek cigarettes relative to American-blended cigarettes, two commercial Indonesian brands and an experimental kretek cigarette representative of the average kretek cigarette on the Indonesian market were evaluated against the American-blended reference cigarette 2R4F. This assessment was performed through both chemical characterization of the MS and an *in vitro* determination of MS total particulate matter (TPM) mutagenicity as well as of gas/vapor-phase (GVP) and TPM cytotoxicity.

2. Materials and methods

2.1. American-blended cigarette selection

The University of Kentucky reference cigarette 2R4F, which is widely used as both a reference and a monitor cigarette, was selected in this study as the American-blended cigarette benchmark for kretek cigarette comparisons. The design and blend

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composition of the reference cigarette 2R4F were selected to be representative of the most popular products on sale in the US cigarette market (Chen and Moldoveanu, 2003; Roemer et al., 2012b). The reference cigarette 2R4F filler is a blend of flue-cured (32.5%), Burley (19.9%), Oriental (11.1%), Maryland (1.2%) and reconstituted (27.1%) tobaccos, with addition of 5.3% of inverted sugars and 2.8% of glycerin (Chen and Moldoveanu, 2003; Roemer et al., 2012b).

Different versions of the R4F series of reference cigarettes (1R4F, 2R4F and 3R4F) were produced as supplies were depleted, but differences among these versions were shown to be minimal (Counts et al., 2006; Roemer et al., 2012b). On the basis of chemical analyses and *in vitro* mutagenicity assays, it was reported in 2000 that the 1R4F version was for the full flavor low tar segment an "*acceptable reference cigarette*(*s*) *for comparative mutagenicity and smoke chemistry studies of cigarettes available on the US market*" (Chepiga et al., 2000). The basic design elements have remained similar over recent years among brands in the US market and, on average, the yields of the monitored smoke constituents from representative US brands have changed very little over time (Bodnar et al., 2012; Swauger et al., 2002). It can thus be concluded that the 2R4F reference cigarette remains representative of US commercial brands with International Organization for Standardization (ISO) deliveries of around 10 mg tar per cigarette (Counts et al., 2006). The reference cigarette 2R4F was obtained from the University of Kentucky (Kentucky Tobacco Research & Development Center).

2.2. Kretek cigarettes selection

A kretek reference cigarette was designed and manufactured in 2005 by PT Hanjaya Mandala Sampoerna Tbk. to be representative of the most popular kretek cigarettes on sale on the Indonesian market, following the same logic as the one that led to the development of the 2R4F cigarette for use as a reference and a monitor product representative of American-blended cigarettes in comparative studies. It is referred to as the kretek reference cigarette (abbreviated as Kretek-R).

In addition to the Kretek-R, two commercial kretek cigarette brands were selected from the portfolios of Gudang Garam Tbk. and Hanjaya Mandala Sampoerna Tbk., the two largest Indonesian kretek cigarette manufacturers (Arnez, 2009), to represent the wide range in designs that prevails among machine-made kretek cigarettes on the Indonesian market.

The *Gudang Garam International Filter* cigarette (thereafter referred to as Garam), the leading brand of PT Gudang Garam Tbk., was, at the time of the sampling, the best-selling Indonesian brand. Garam is representative of the high-tar segment of machine-made kretek cigarettes; it is slightly larger in diameter than American-blended cigarettes and is fitted with a short, non-ventilated filter.

The *A Mild 16* cigarette (thereafter referred to as Sampoerna) was chosen as being representative of the lower-tar segment of machine-made kretek cigarettes on the Indonesian market. This cigarette is longer and slimmer than the American-blended reference cigarette 2R4F and most commercial kretek cigarette brands, and is fitted with a ventilated filter. At the time of the sampling, this cigarette was the leading brand of PT Hanjaya Mandala Sampoerna Tbk.

2.3. Analytical methods

Detailed descriptions regarding the chemical analyses, toxicological assays and statistical procedures can be found in the first of this series of publications (Roemer et al., 2014c). Briefly, smoke was generated from all test cigarettes according to ISO standards 3308 and 4387 (ISO-3308, 2000; ISO-4387-4, 2000). This smoking regimen was choosen, as for kretek cigarettes with a yield of up to 50 mg ISO tar the application of more intense smoking regimens would lead to estimated tar yields of far more than 100 mg per cigarette. It seems rather unlikely that the actual intake of a smoker comes near to such values.

Chemical analyses were performed on TPM and the GVP fractions. The methods used for each of the analytes are detailed in the lead publication (Roemer et al., 2014c). The Neutral Red Uptake (NRU) assay was used to assess the TPM and GVP cytotoxicity, as detailed previously (Bombick et al., 1998; Roemer et al., 2002). As a measure of effect, the inverse of the calculated EC_{50} is reported. To assess the TPM mutagenicity, the *Salmonella* Reverse Mutation Assay (Maron and Ames, 1983) was performed on the TPM from each cigarette using five tester strains of *Salmonella typhimurium*, both with and without a metabolic activation system (S9).

Table 1
Design parameters of the cigarettes used for testing: *Gudang Garam International* (Garam), *A Mild 16* (Sampoerna), kretek reference cigarette (Kretek-R), and reference cigarette 2R4F.

Design parameter (unit)	Garam	Sampoerna	Kretek-R	2R4F
Cigarette weight (mg at 12.5% moisture)	1370	985	1420	1055
Circumference (mm)	25.4	22.0	24.9	24.9
Cigarette length (mm)	80.0	90.0	89.9	83.9
Tobacco column length (mm)	65.0	65.0	70.0	57.0
Filler weight (mg at 12.5% moisture)	1187	774	1200	810[a]
Estimated clove content (%)	33	31	31	–
Tipping paper length (mm)	19.0	31.0	25.0	32.0
Paper porosity (Coresta units)	30	55	60	24[a]
Cigarette filter ventilation (%)	0	40	1.5	28
Cigarette pressure drop (mm water)	95	118	138	138

[a] From published data (Carmines, 2002; Davies and Vaught, 2003).

3. Results

3.1. Tobacco analyses

According to the manufacturer, the design of the Kretek-R cigarette was based on a filler made from a blend of 55% Krosok and 45% Rajagan tobaccos, with addition of dried cloves to reach a 31% level in the final blend. Humectants were added as processing aids to the tobacco and cut clove blend at a level of 4.5%. Tobacco filler samples from the commercial kretek cigarettes were subjected to optical screening of the blend components to assess their blend design. The tobacco used in the filler of both kretek cigarettes is also likely to be a blend of Indonesian sun-cured tobacco with more widely used tobacco types such as flue-cured and Oriental. Neither brand appeared to contain reconstituted tobacco. As expected for a lower-delivery product, the Sampoerna cigarette contained about 7% of expanded tobacco[1] but the Garam cigarettes did not. The clove incorporation level in the filler of the Sampoerna cigarette was 31%.[2] A comparative summary of the key design parameters of the four tested cigarettes is given in Table 1.

Both kretek cigarettes were further characterized by measuring the eugenol content of the filler. From the measured eugenol levels, the proportion of cut cloves in the Garam cigarettes was estimated to be somewhat higher than in the Sampoerna, in the range of 31–35%. The results of the colorimetric analyses regarding the levels of ammonia–nitrogen, reducing sugars, and total alkaloids in the tobacco filler are listed in Table 2, expressed as percent of the filler dry weight. Also shown in Table 2 are the measured filler levels of

[1] Information obtained from the manufacturer.
[2] Information obtained from the manufacturer.

Table 2
Cigarette filler analytical data, on a dry-weight basis (DWB).

Parameter	Unit	Garam	Sampoerna	Kretek-R	2R4F
Total alkaloids	%, DWB	1.33	1.29	1.77	2.31[a]
Reducing sugars	%, DWB	12.4	12.8	11.5	10.7[a]
Total sugars	%, DWB	14.8	13.9	13.1	NA
Ammonia–nitrogen	%, DWB	0.04	0.03	0.04	0.16[b]
Nitrate–nitrogen	%, DWB	0.05	0.06	0.06	0.97[b]
Eugenol	%, DWB	4.18	3.75	3.87	Trace[c]
Eugenol acetate	%, DWB	0.50	0.67	0.56	0
β-Caryophyllene	%, DWB	0.46	0.45	0.49	0

Abbreviations: Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*, Kretek-R, *kretek reference cigarette*, NA: *Not available.*
[a] From published data (Chen and Moldoveanu, 2003).
[b] From published data (Counts et al., 2006).
[c] Trace amounts (tens of ppm) are present since about 1 μg/cigarette was detected in smoke.

Table 4
Transfer rates of nicotine and eugenol to MS and their ratio.

Parameter	Unit	Sampoerna	Garam	Kretek-R
Filler weight	mg/cig	774	1187	1230
Alkaloid in filler	mg/cig	8.8	13.9	19.2
Eugenol in filler	mg/cig	18.3	43.2	35.4
Nicotine smoke delivery	mg/cig	0.80	1.79	1.72
Eugenol smoke delivery	mg/cig	2.52	8.26	4.79
Nicotine transfer[a]	%	8.01	11.3	9.0
Eugenol transfer[a]	%	13.8	19.1	13.5
Ratio of eugenol transfer to nicotine transfer[a]		1.52	1.48	1.51

Abbreviations: Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*.
[a] From data sets dedicated to smoke transfer assessments.

major components of the clove essential oil, i.e., eugenol, eugenol acetate and β-caryophyllene for each of the kretek cigarettes tested in the study.

3.2. Smoke chemistry

3.2.1. ISO parameters and eugenol

An average of the ISO carbon monoxide (CO), nicotine and tar yields of the tested cigarettes are shown in Table 3. The reference cigarette 2R4F was smoked concurrently with each kretek cigarette analyzed. Overall, averaged yields of 9.9 mg/cigarette of tar and 12.7 mg/cigarette of CO based on a puff count of 9.5 were observed, which is in good agreement with published data (Davies and Vaught, 2003).

When reporting ratios of individual kretek cigarettes deliveries to American-blended cigarettes deliveries, the calculation was performed using the reference cigarette 2R4F data generated concurrently with the kretek cigarette analysis. Reflecting the diluting effect of cut cloves in the cigarette filler which contribute to the TPM but do not yield any nicotine, the nicotine deliveries of the kretek cigarettes were respectively 39%, 29% and 46% lower than would be expected if they had had the same tar/nicotine ratio as the reference cigarette 2R4F. Transfer rates of eugenol in Sampoerna, Garam and the Kretek-R cigarette were determined, together with those of nicotine and tar, in additional dedicated smoking runs. The transfer rates for eugenol in Sampoerna, Garam and the Kretek-R cigarette were determined to be 15.7%, 17.8% and 14.3% of the total TPM delivery, respectively (Table 4).

3.2.2. Assessment by classes of compounds

For all measured MS constituents, the smoke delivery of each kretek cigarette and the reference cigarette 2R4F expressed on a per cigarette basis are given in Table A of the Appendix. In all cases, the differences between the kretek cigarettes and the reference cigarette 2R4F deliveries were statistically significant.

As discussed in the lead publication, results should be normalized for a meaningful comparative assessment of smoke composition and *in vitro* toxicity to be performed when comparing cigarettes with very different designs and delivery ranges. In the present situation a normalization by TPM was deemed most appropriate (Roemer et al., 2014c). The relative difference of the constituents yields compared to the deliveries of reference cigarette 2R4F when smoked in the same analytical batch, and normalized by TPM yield, are presented in Table 5. The significance of the observed percent changes relative to the reference cigarette 2R4F delivery, when measured concomitantly with the kretek cigarette determinations and normalized by TPM yield, was assessed by analysis of variance (ANOVA) followed by a Dunnett's test as detailed in the lead publication (Roemer et al., 2014c). The results of all statistical tests on the differences are also reported in Table 5.

For visualization of the changes by class of compounds, the TPM-normalized data in Table 5 are displayed in the form of a radar chart (Fig. 1A). The scales on the chart are logarithmic so that the large range of values (almost 2 orders of magnitude) can be represented while ensuring that data points are spaced proportionally to their ratio (e.g., 200% and 50% are equally spaced from 100%). For comparison, the results obtained when the normalization is performed to the nicotine yield instead of the TPM are shown in Fig. 1B.

Overall, the MS delivery of polycyclic aromatic hydrocarbon (PAH) compounds were at similar levels in the smoke of all cigarettes, with very few significant differences, after TPM normalization. The levels of dihydroxybenzenes were also similar, although differences among products were larger than in the case of PAH compounds; catechol was found at significantly higher concentrations in Sampoerna than in the reference cigarette 2R4F, and hydroquinone at significantly lower concentrations in Garam and Kretek-R than in the reference cigarette 2R4F. Formaldehyde was at similar levels in the smoke from all cigarettes

Table 3
MS yields per cigarette; International Organization for Standardization machine smoking regimen; mean, standard error (S.E.) and number of determinations (*N*).

Parameter (unit)	Sampoerna		Garam		Kretek-R		2R4F	
	Mean	S.E. (*N*)	Mean	S.E. (*N*)	Mean	S.E. (*N*)	Mean	S.E. (*N*)
Puff count (*n*)	12.6	0.1 (4)	17.3	0.1 (4)	14.4	0.1 (4)	9.5	0.1 (4)
TPM (mg/cig)	16.1	0.1 (4)	46.4	0.8 (4)	33.6	0.2 (4)	12.0	0.1 (4)
Tar (mg/cig)	14.4	0.2 (4)	39.9	0.7 (4)	29.0	0.1 (4)	9.91	0.12 (4)
Nicotine (mg/cig)	0.74	0.04 (4)	1.78	0.02 (4)	1.72	0.02 (4)	0.829	0.012 (4)
Water (mg/cig)	0.928	0.029 (4)	4.81	0.17 (4)	2.86	0.05 (4)	1.27	0.05 (4)
Carbon monoxide (mg/cig)	8.98	0.34 (4)	17.8	0.8 (6)	18.7	0.2 (4)	12.7	1.0 (4)

Abbreviations: Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*, TPM, total particulate matter.

Table 5
Garam, Sampoerna and Kretek-R smoke constituents ISO yields normalized to TPM as percent of 2R4F (100%); mean, standard deviation (S.D.) and statistical significance of difference with 2R4F.

Parameter[a]	Garam			Sampoerna			Kretek-R		
	Mean (%)	S.D. (%)	Signif.	Mean (%)	S.D. (%)	Signif.	Mean (%)	S.D. (%)	Signif.
ISO parameters									
TPM	100			100			100		
Tar	104	3	*	109	2	***	105	1	*
Nicotine	56	2	***	66	1	***	69	1	***
Water	98	6		55	3	***	84	3	**
Carbon monoxide	36	3	***	53	5	***	48	1	***
Aliphatic dienes									
1,3-Butadiene	50	5	***	69	10	***	68	3	***
Isoprene	28	3	***	42	3	***	39	1	***
Aldehydes									
Formaldehyde	94	10		155	15	***	100	3	
Acetaldehyde	26	2	***	47	3	***	40	1	***
Acrolein	28	3	***	58	6	***	51	2	***
Propionaldehyde	36	3	***	54	4	***	46	1	***
Aliphatic nitrogen compounds									
Acetamide	93	5	*	77	7	***	74	2	***
Acrylonitrile	39	3	***	53	5	***	54	1	***
2-Nitropropane	16	2	***	25	3	***	29	1	***
Aromatic amines									
o-Toluidine	56	4	***	66	4	***	66	1	***
o-Anisidine	46	4	***	56	4	***	47	1	***
2-Naphthylamine	45	5	***	49	6	***	61	2	***
4-Aminobiphenyl	42	5	***	42	5	***	57	2	***
Halogen compounds									
Vinylchloride	52	4	***	54	7	***	49	2	***
Inorganic compounds									
Nitrogen oxides	17	1	***	25	2	***	17	1	***
Hydrogen cyanide	57	4	***	58	4	***	63	2	***
Monocyclic aromatic hydrocarbons									
Benzene	45	2	***	60	5	***	54	1	***
Toluene	40	2	***	49	4	***	46	1	***
Styrene	60	5	***	62	6	***	61	2	***
N-nitrosamines									
NNN	9	1	***	12	2	***	8	1	***
NNK	5	1	***	13	2	***	5	1	***
Phenols									
Phenol	227	10	***	247	11	***	176	3	***
Catechol	100	7		114	7	**	101	2	
Hydroquinone	66	8	***	93	13		82	2	***
Polycyclic aromatic hydrocarbons									
Benz[*a*]anthracene	117	12	**	114	9	*	104	2	
Benzo[*b*]fluoranthene	96	4		113	8	**	95	2	**
Benzo[*j*]fluoranthene	99	5		110	4	**	104	2	
Benzo[*k*]fluoranthene	IQD			IQD			101	2	
Benzo[*a*]pyrene	95	3	*	103	5		102	3	
Indeno[1,2,3-*cd*]pyrene	74	6	***	103	8		102	2	
Dibenz(a,h)anthracene	IQD			IQD			IQD		
Dibenzo[*a,e*]pyrene	IQD			IQD			IQD		
Dibenzo[*a,h*]pyrene	IQD			IQD			IQD		
Dibenzo[*a,i*]pyrene	IQD			IQD			IQD		
Dibenzo[a,I]pyrene	IQD			IQD			IQD		
5-Methylchrysene	IQD			IQD			IQD		
Elements									
Arsenic	IQD			IQD			54	3	***
Cadmium	41	3	***	22	1	***	75	2	***
Chromium	IQD			IQD			IQD		
Nickel	IQD			IQD			IQD		
Lead	76	6	***	57	4	***	61	2	***

Significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (p-value from analysis of variance followed by Dunnett's test).
[a] *Abbreviations:* NNN, N′-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; TPM, total particulate matter; ISO, International Organization for Standardization; IQD: Incomplete quantitative data; Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*.

except for Sampoerna, where a significant increase of 55% was observed. Except acetamide and phenol, the remaining compounds were present in the MS of kretek cigarettes at less than half of the levels determined in the reference cigarette 2R4F; these differences were statistically significant. An even larger reduction was observed for nitrogen-containing compounds. Volatile nitrosamines were not detected and tobacco-specific N-nitrosamines (TSNA) were only found in the MS of kretek cigarettes at levels more than 10 times lower than those found for the reference cigarette 2R4F. Nitrogen oxides deliveries in kretek cigarettes were 17–25% of the levels found in the reference cigarette 2R4F. When normalization by nicotine is considered, data are multiplied by 1.85, 1.69 and 1.46 for Garam, Sampoerna and Kretek-R, respectively, essentially reflecting the respective percentages of clove addition which in all cases amounts to about 1/3 by mass of the total filler material.

3.2.3. Product evolution over time

The studies described in this publication were performed in 2003 on commercial cigarettes obtained at this point in time (set 1), although the studies described in the other publications on the toxicity of kretek cigarettes, to be found in the same supplement of this journal, are more recent. To assure that the commercial products characterized in this publication are still comparable to those currently marketed, we compared our smoke emission data for one of the studied brands (Sampoerna) to those obtained in 2013 by the Hanjaya Mandala Sampoerna Tbk. laboratory (set 2) from current cigarettes with the same brand name. Nineteen of the smoke analytes presented in this publication had been quantified, and for these the data were compared with an *x*/*y* plot.

It is obvious that this comparison is distorted to some extent by the fact that the compared analytical data were obtained using quite different instrumentation and analytical methods. Nevertheless, the direct comparison of the analytes yields on a per cigarette basis gave a rather tight linear correlation characterized by an R^2 of 0.887. To account for the differences in methodologies, each analyte yield in set 1 was adjusted using the ratio of the yields obtained for the 2R4F reference cigarette for the same analyte under the conditions of each set (factor: 2R4F set 2/2R4F set 1). After such adjustment, the correlation could be improved even further. The results of the comparison are shown on Fig. 2, displayed on a $\log_{10}/\log_{10}$ scale to account for the wide span of the data; the high R^2 of

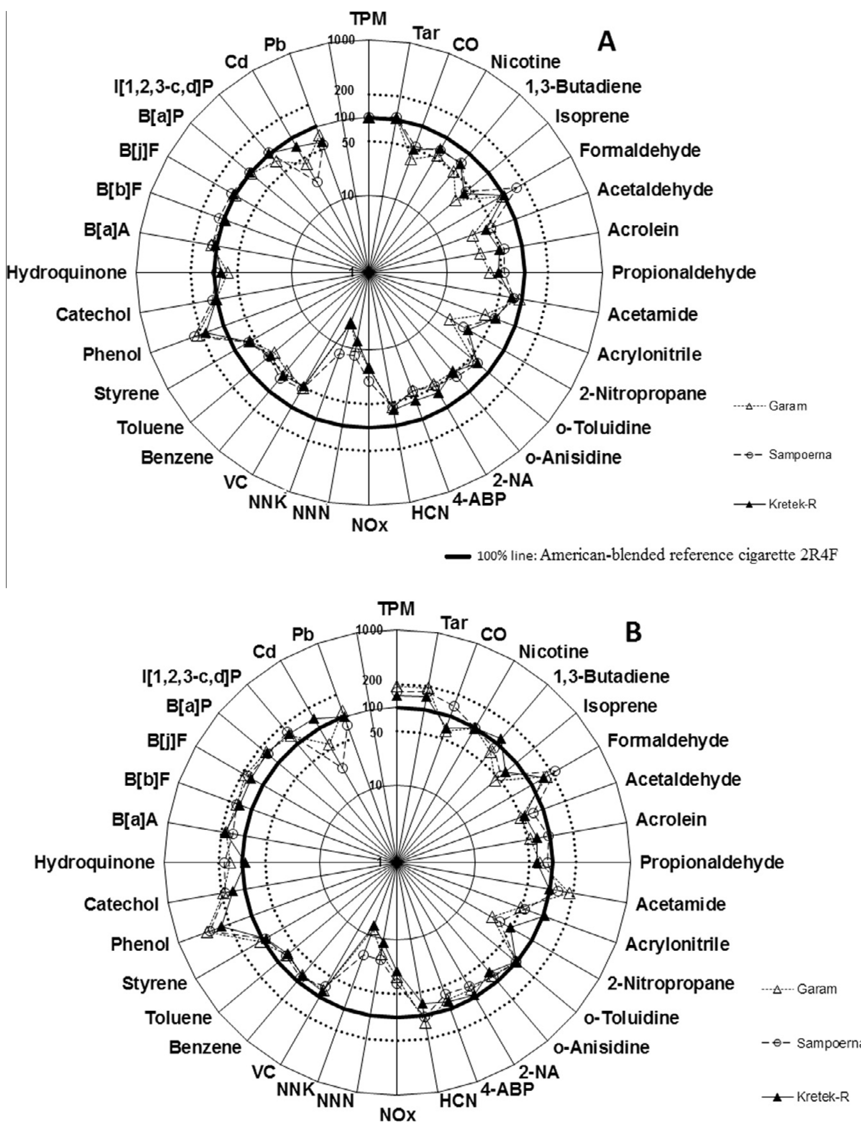

Fig. 1. MS yields of kretek cigarettes (ISO machine smoking regimen), as percentages of 2R4F yields, all normalized by TPM (A) and nicotine (B); ($\log_{10}$ scales). All deliveries of the kretek cigarettes relative to the 2R4F are statistically different, except in some cases for formaldehyde, and dihydroxy-benzenes, for data see text and Table 5. *Abbreviations:* NNN, N′-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; B[a]A, Benz[a]anthracene; B[b]F, Benzo[b]fluoranthene; B[j]F, Benzo[j]fluoranthene; B[a]P, Benzo[a]pyrene; I[1,2,3-c,d]P, Indeno[1,2,3-c,d]pyrene; Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*; TPM, total particulate matter; NOx, nitrogen oxides; 2-NA, 2-aminonaphthalene; 4-ABP, 4-aminobiphenyl; VC, vinylchloride; ISO, International Organization for Standardization.

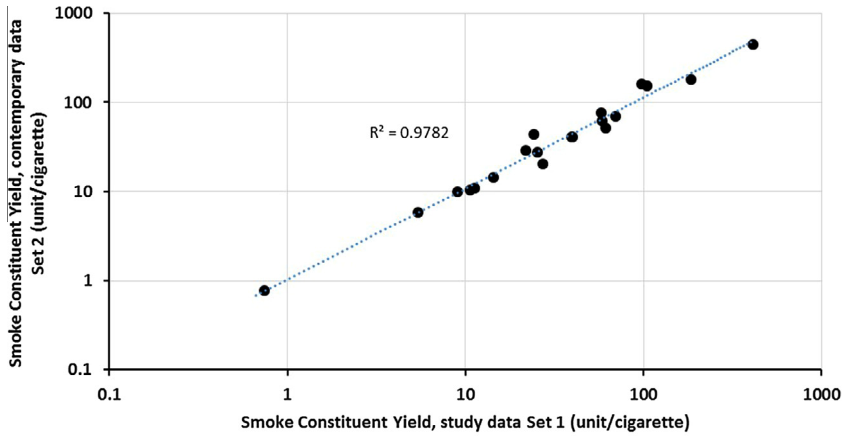

Fig. 2. Comparison of smoke constituent yields for the Sampoerna kretek cigarette obtained in 2003 (set 1) and in 2013 (contemporary data, set 2), measured in two laboratories. To account for differences in methodologies, contemporary data were adjusted based on the ratio of yields obtained for the 2R4F reference cigarette analyzed in both laboratories ($\log_{10}$ scales).

Table 6
Cytotoxicity of TPM and GVP, expressed as $1/EC_{50}$ on a mg TPM basis.

$1/EC_{50}$[a] (ml/mg TPM) $N = 3$	Sampoerna		Garam		Kretek-R		2R4F	
	TPM	GVP	TPM	GVP	TPM	GVP	TPM	GVP
Mean	11.41	6.18	9.08	3.52[*]	10.42	4.13[*]	10.33	8.26
Std. error	1.33	0.67	0.74	0.09	0.38	0.10	0.41	0.37
RSD (%)	20.1	18.7	14.1	4.5	6.3	4.1	6.9	7.7

[*] Statistically different from 2R4F data, $p \leqslant 0.001$ (p-value from two-tailed t-test).
[a] *Abbreviations:* Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*; TPM, total particulate matter; GVP, gas–vapor phase; RSD, relative standard deviation; EC_{50}, effective concentration 50, a concentration of a substance expected to cause the reduction of an entire defined experimental cell population by 50%; N, number of determinations.

0.978 points to the high similarity of our data with those of the current market products. It has to be noted that the establishment of the analysis methods for set 2 had been done with the information of the standard operation methods of the first laboratory.

3.3. In vitro toxicity

3.3.1. Cytotoxicity

Cytotoxicity data for both the GVP and TPM fractions, expressed as $1/EC_{50}$ on a per mg TPM basis, are provided in Table 6 for all three kretek cigarettes as well as the reference cigarette 2R4F.

The cytotoxicity of the TPM on a per mg TPM basis was similar in all cigarettes, and none of the observed differences were statistically significant. Larger differences were found among the samples obtained from the GVP. As a quality assessment, the sampling of the GVP batch was assessed by the measurement of the trapped TPM and the concentration of acrolein trapped in the sampling buffer solution. The sampling was found to be reproducible (SD below 5% of the mean).

More pertinent for discussion are the data expressed as percentages of the reference cigarette 2R4F cytotoxicity data. In this case, the ratio was computed using the 2R4F determinations obtained in the same analytical batch as the studied kretek cigarette. The cytotoxicity of both GVP and TPM fractions of the MS of all three kretek cigarettes is shown in Fig. 3, expressed as percentage of the 2R4F cytotoxicity.

Fig. 3 shows that the cytotoxicity of the GVP from the kretek cigarettes was lower than that of the reference cigarette 2R4F on a per mg TPM basis. Compared to the 2R4F, the observed difference was statistically significant in the case of the Garam (41% of 2R4F) and Kretek-R (50% of 2R4F), but not in the case of the Sampoerna (71% of 2R4F).

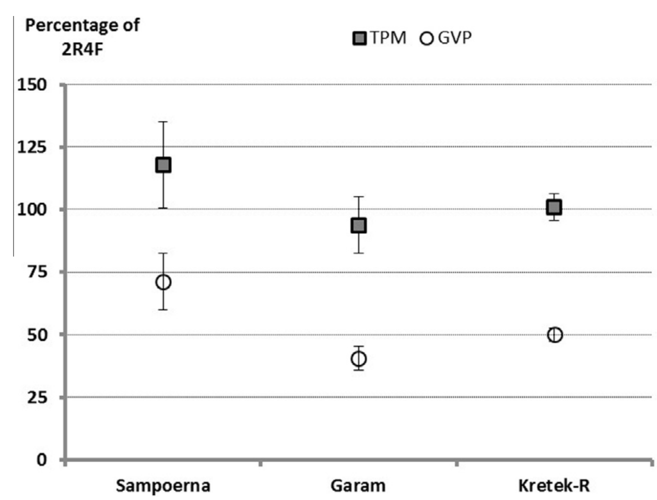

Fig. 3. Cytotoxicity of TPM and GVP from all kretek cigarettes, normalized by TPM ($1/EC_{50}$ in ml/mg), shown as percentage of that of 2R4F, with bars displaying the standard error. *Abbreviations:* Garam, *Gudang Garam International Filter*; Sampoerna, A Mild 16; Kretek-R, kretek reference cigarette; EC_{50}, effective concentration 50, a concentration of a substance expected to cause the reduction of an entire defined experimental cell population by 50%; TPM: total particulate matter; GVP, gas/vapor phase.

3.3.2. Bacterial mutagenicity

S. typhimurium tester strains TA98, TA100, and TA1537, both with and without S9 activation, were always responsive to exposure to TPM from both the reference cigarette 2R4F and Kretek-R. In contrast, the response observed from tester strains TA102 and

Table 7
Bacterial mutagenicity of TPM from all cigarettes expressed both as revertants per mg TPM and as percentage of the 2R4F mutagenicity. Means and standard error (SE) for the 3 responsive strains with and without metabolic activation (S9).

Bacterial strains		Revertants per mg TPM[a]								As percentage of 2R4F (%)-per mg TPM					
		2R4F		Sampoerna		Garam		Kretek-R		Sampoerna		Garam		Kretek-R	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
+S9	TA98	3450	83	1423	76	1295	54	861	55	41	2	38	2	41	3
	TA100	1281	63	656	75	519	47	498	62	51	6	41	4	50	8
	TA1537	381	21	149	25	178	20	174	27	39	7	47	6	47	8
−S9	TA98	25	3	–[b]	–[b]	10	2	9	2	–[b]	–[b]	40	9	64	20
	TA100	123	13	81	17	90	13	82	13	66	15	73	13	115	32
	TA1537	10	2	–[b]	–[b]	5	1	5	2	–[b]	–[b]	50	14	33	15

Note that the 2R4F data effectively used for the calculation of the percentages in Fig. 4 and Table 7 were those acquired from the reference cigarette 2R4F concurrently with each of the respective kretek cigarettes.
[a] *Abbreviations:* Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*; TPM, total particulate matter; S9, metabolic activation (supernatant after centrifugation of liver homogenate from rats treated with Aroclor 1254).
[b] Significant activity increase observed without S9 for TA98 and TA1537, but no quantifiable dose–effect could be derived.

TA1535 did not allow any reliable quantification, consistent with previous studies on cigarette smoke mutagenicity (Baker et al., 2004; Roemer et al., 2008, 2002). Furthermore, in the absence of metabolic activation by S9, tester strains TA98 and TA1537 did not exhibit statistically significant dose-dependent increases in the number of revertants when TPM from Sampoerna was assayed. The mean mutagenic activity towards the three responsive tester strains (TA98, TA100 and TA1537), with and without S9 metabolic activation, are shown as revertants per mg TPM in Table 7 for the three kretek cigarettes, together with the values obtained from reference cigarette 2R4F. More pertinent for discussion are the data expressed as percentages of the 2R4F activity, also reported in Table 7. In this case, the ratio was computed using the 2R4F values obtained in the same analytical batch as the studied kretek cigarette. These percentages are shown in Fig. 4.

With addition of S9 metabolic activation, the specific mutagenicity of the TPM from all kretek cigarettes using all tester strains was 40–50% of the specific mutagenicity of the 2R4F cigarette. This difference was in all instances statistically significant. Without S9, the activity of the TPM from all tested cigarettes was much lower, with tester strain TA100 giving the highest response. For this strain, the specific mutagenic activity of TPM from kretek market brands was about 70% of the American-blended cigarette activity, but it was 15% higher than the American-blended cigarette activity for Kretek-R (none of the differences between kretek cigarettes and the reference cigarette 2R4F were significant).

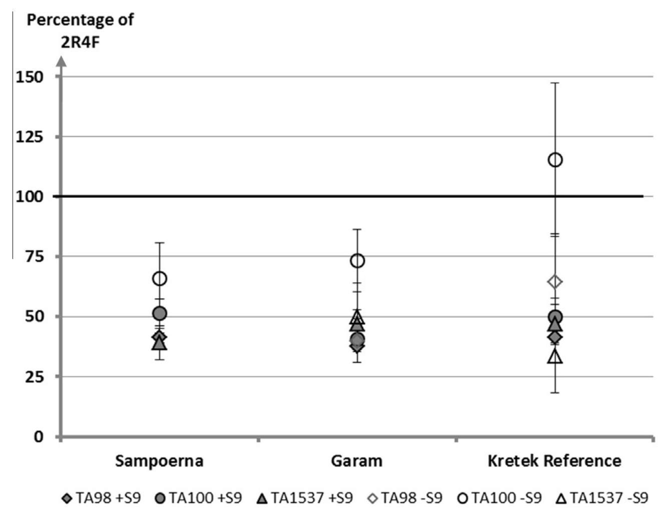

Fig. 4. Bacterial mutagenic activity per mg TPM for 3 tester strains of TPM from all kretek cigarettes, plotted as percentage of the corresponding American-blended 2R4F activity, with (filled markers) and without (open markers) S9 metabolic activation. Bars display the standard error. In the case of Sampoerna, significant activity increases were observed without S9 for TA98 and TA1537 but no quantifiable dose–effect could be derived. *Abbreviations:* Garam, *Gudang Garam International Filter*; Sampoerna, A Mild 16; Kretek-R, kretek reference cigarette; TPM, Total particulate matter; S9, metabolic activation (supernatant after centrifugation of liver homogenate from rats treated with Aroclor 1254).

4. Discussion

4.1. Chemical analyses

Eugenol levels in the cigarette fillers were consistent with the upper levels reported in a study on 33 brands of clove-flavored cigarettes from 5 kretek cigarette manufacturers (Polzin et al., 2007). The levels of the other major clove essential oil components (β-caryophyllene, eugenol and eugenol acetate shown in Table 2) are also consistent with levels reported in a previous study (Musenga et al., 2006). As shown in Table 4, the transfer rates of nicotine and eugenol to the MS of each kretek cigarette are in about the same ratio in all tested kretek cigarettes. This suggests that in kretek cigarettes the transfer of nicotine and eugenol to the MS are influenced in a similar way by the design of the cigarettes.

When the smoke composition of kretek cigarettes is compared to that of American-blended reference cigarette 2R4F on an equal TPM basis, a major difference is the lower delivery of most of the nitrogen-containing compounds. This is a direct consequence of the much lower nitrogen contents of the types of tobacco used in kretek cigarette fillers, compared to the filler of the reference cigarette 2R4F (the ammonium ion levels are about 4 times lower and nitrate ion levels are about 20 times lower than in the reference cigarette 2R4F, see Table 2). The yields of aromatic amines and low molecular weight nitrogen-containing compounds represented by 2-nitropropane, acrylonitrile and nitrogen oxides are, in the MS of all kretek cigarettes, about half of the levels found for the reference cigarette 2R4F or less. The yield reductions are particularly pronounced in the case of TSNA with yields more than 10 times lower than the reference cigarette 2R4F. Such reduction is much larger than what would be anticipated from the mere replacement of about one third of the tobacco filler weight with dried clove buds. This may be due to lower levels of preformed TSNAs in the tobacco part of the kretek cigarette filler than in the American-blended filler.

Short-chain aldehydes are delivered in substantially lower amounts per mass of TPM in the smoke from kretek cigarettes than from the reference cigarette 2R4F. For instance, the respective normalized deliveries from Garam, Sampoerna and Kretek-R cigarettes are only about 30%, 60% and 50% of the reference cigarette 2R4F for the delivery of acrolein, and about 25%, 45% and 40% for acetaldehyde. Short-chain aldehydes in smoke do not share a common formation pathway (Piadé et al., 2013; Sanders et al., 2002). They are considered to be formed, in part, by the decomposition of carbohydrates or their derivatives (Baker, 1999, 2006; Sanders et al., 2002), but in the case of acetaldehyde the main precursors have not been fully identified (Teillet et al., 2012). The yields of most aldehydes after normalization to TPM are lower in both kretek cigarettes compared to the reference cigarette 2R4F, suggesting that upon burning, cut cloves contribute less to the yield of aldehydes than tobacco filler does, or possibly inhibit the delivery of aldehydes. This is consistent with the findings of Roemer et al. (2014b). In contrast with other aldehydes, the delivery of formaldehyde normalized to TPM is either 55% higher (Sampoerna), 5% lower (Garam), or unchanged (Kretek-R) compared to the yield of the reference cigarette 2R4F. The fact that the yield reduction is lower than in the case of the other aldehydes may be linked to the similar levels of mono- and disaccharides observed in the filler of all cigarettes; deliveries of these compounds have been shown to be correlated with formaldehyde yield (Cahours et al., 2012; Roemer et al., 2012a). Moreover, kretek cigarettes have much lower nitrogen contents than the reference cigarette 2R4F, and elevated formaldehyde yields are usually observed in the smoke of products with low nitrogen contents (Piadé et al., 2013).

The PAH yields were essentially the same in all investigated cigarettes when normalized to TPM. These compounds are generated from a very broad set of precursors common to biomass products of all kinds (Ding et al., 2006; Piadé et al., 2013), so that large changes would not be expected in deliveries normalized to TPM provided that pyrolysis conditions would be similar in clove and tobacco materials (Rodgman, 2001). Nitrate levels have been reported to be negatively correlated with PAH smoke deliveries (Ding et al., 2006; Hoffmann and Hoffmann, 2001), but the effect of the low nitrate contents in kretek articles was not sufficient to result in increased PAH yields normalized to TPM.

The yields of catechol and hydroquinone are not increased in kretek cigarettes. However, the phenol delivery is noticeably larger in the MS of kretek cigarettes than in that of the reference cigarette 2R4F. This does not appear to be a consequence of the presence of clove or eugenol in the kretek cigarettes, since phenol is a very minor product of eugenol pyrolysis (Fitzpatrick et al., 2007). It could be the result of the tobacco treatment.

The commercial kretek cigarettes exhibit substantially lower cadmium and lead MS deliveries than the reference cigarette 2R4F, while no arsenic, chromium or nickel could be detected. Although no filler data are available, this result is the likely consequence of the level of the various elements in the cigarette. Notably, metals and arsenic in tobacco have been shown to vary substantially depending on its origin (Lugon-Moulin et al., 2006).

Normalization to nicotine has been recommended by authoritative bodies, e.g., World Health Organization (WHO, 2009) and individual researchers (Rickert et al., 2007, 2011; Ashley et al., 2008). Since tobacco is the only source of smoke nicotine, the replacement of about one third of the tobacco filler weight with dried clove buds has a direct impact when assessing the data after normalization by nicotine. When the kretek cigarettes normalized yields are expressed as a percentage of the corresponding normalized yield of the reference cigarette 2R4F, data are about one and a half time higher than the TPM-normalized data, essentially reflecting the respective percentages of clove addition. Obviously the pattern of differences among the monitored compounds remains the same and the above discussion on TPM-normalized data remains valid. However, in the comparison with the 2R4F, the difference changes. The smoke yields of nitrogen-containing constituents, aldehydes and volatile aromatic compounds remain, in most cases, lower for kretek cigarettes than for the American-blended 2R4F. The exceptions are formaldehyde and acetamide. The dihydroxy benzenes and PAHs smoke yields are in this case about 50% higher than for the 2R4F, and phenol yield is about 3 times higher.

4.2. In vitro toxicity

4.2.1. Mammalian cell cytotoxicity

Both GVP and TPM fractions of cigarette smoke display a significant inherent cytotoxic activity. With regard to the TPM, no statistically significant differences in cytotoxicity were observed in kretek cigarettes and the reference cigarette 2R4F. The nature of the major contributors to TPM cytotoxicity is still uncertain. It has been suggested that aldehydes and alkyl alcohols, indole derivatives and oxygen-containing terpenoids, all compounds that have been individually tested as highly toxic, could be important contributors (Curvall et al., 1984). More recent studies concluded that hydroquinone and catechol were major contributors, while nicotine does not appear to have a notable effect (Richter et al., 2010). On the basis of these studies, the similarity of TPM cytotoxicity in kretek cigarettes and the reference cigarette 2R4F is consistent with the smoke chemistry assessment. Notably, the smoke levels of hydroquinone and catechol are similar for all tested cigarettes on a per mg TPM basis.

In contrast to the TPM, the cytotoxicity elicited by GVP smoke components was found to differ among the tested cigarettes. The cytotoxicity of the GVP from the kretek cigarettes was 30% (Sampoerna), 50% (Kretek-R) and 60% (Garam) lower than that from the reference cigarette 2R4F; the differences were statistically significant ($p \leqslant 0.001$) in the cases of Kretek-R and Garam. Acrolein is a known cytotoxic agent in cigarette smoke (Comer et al., 2014; DeWoskin et al., 2003; Green, 1985; van der Toorn et al., 2013) which provides an important contribution to the response of smoke GVP in the NRU cytotoxicity assay (Tewes et al., 2003). Acrolein levels (normalized to TPM) were measured at 58–28% of the levels found in the reference cigarette 2R4F (Fig. 1 and Table 5), in concordance with the ranking observed from the NRU assay. Crotonaldehyde, a methylated derivative of acrolein that comprises the same α,β-unsaturated aldehyde structure, has also been associated with smoke cytotoxicity (Cheah et al., 2013; van der Toorn et al., 2013). The crotonaldehyde yield in the Kretek-R, was 68% of the level in the reference cigarette 2R4F. In fact, smoke deliveries of all volatile aldehydes (normalized to TPM) from the kretek cigarettes were found to be lower than from the reference cigarette 2R4F, 40–50% lower in the case of Sampoerna and Kretek-R, and about 70% lower in the case of Garam. The formaldehyde deliveries, however, were found to be similar for the kretek cigarettes, in one case higher (Sampoerna). Formaldehyde is also an important gas-phase contributor to smoke cytotoxicity (Lehman-McKeeman, 2010). Altogether, the chemical characterization appears fully consistent with the differences observed in the results of the NRU assay on the GVP fraction.

4.2.2. Bacterial mutagenicity

Similar to the TPM cytotoxicity results, the mutagenicity results from all kretek cigarettes are remarkably similar when data are expressed on a per mg TPM basis. Qualitatively, the similarity of response patterns across all tested strains for all the kretek cigarettes suggests that the TPM specific mutagenicity is very similar among the kretek cigarettes. TA102 and TA1535, tester strains normally insensitive to tobacco smoke TPM, were also insensitive to the TPM of all kretek cigarette brands, which is also consistent with the fact that no new mutagenic activity was observed in the smoke from kretek cigarettes.

Quantitatively, for all tester strains presenting a significant response, i.e., TA98, TA100 and TA1537, the specific mutagenic activity of the TPM from all kretek cigarettes with S9 activation was 40–50% of that of the reference cigarette 2R4F. This reduction is larger than what would be expected from the substitution of one third of the tobacco mass by material that would not contribute any mutagenicity. The lower mutagenic activity measured in kretek TPM was consistent with the observed differences in smoke composition between kretek cigarettes and reference cigarette 2R4F. The lower smoke deliveries for nitrogen compounds (not including nicotine) observed for kretek cigarettes are likely to be an important factor. Nitrogen-containing pyrolysis products, notably from protein pyrolysis, are often cited as important predictors of smoke mutagenicity after enzymatic activation (Shin et al., 2009). In the present case, aromatic amines, which have been reported to correlate well with the total smoke mutagenic activity (Bartsch et al., 1993), are about twice less abundant in the kretek cigarette smoke than in the smoke of the reference cigarette 2R4F. Overall, smoke from Burley or dark tobacco cigarettes, higher in volatile nitrogen compounds, has been shown to be more mutagenic on a TPM basis than smoke from Virginia cigarettes (Belushkin et al., 2014; Rickert et al., 2007; Röper et al., 2004; Schramke et al., 2006). In this respect, kretek cigarette smoke would be even more different from dark tobacco smoke than Virginia tobacco smoke. The PAHs found at comparable levels in MS from kretek cigarettes and the reference cigarette 2R4F, are considered to have little impact on *in vitro* mutagenicity (White et al., 2001). The TSNA smoke deliveries, substantially reduced in kretek cigarettes compared to reference cigarette 2R4F, are considered to only have a very minor impact on the mutagenic activity of smoke condensate (Brown et al., 2001; Doolittle et al., 2000; Yim and Hee, 2001).

5. Conclusions

Despite the diversity in the design characteristics and smoking yields of the selected kretek cigarettes, their smoke chemistry and *in vitro* toxicity were found to be similar when normalized to TPM deliveries. This supports the generality of the conclusions with respect to a wide range of kretek cigarette products.

The smoke composition of kretek cigarettes is characterized by the addition of some clove-specific compounds, notably eugenol. Beyond this, the observed differences in the deliveries of nitrogen-containing compounds, as well as of many of the pyrolytic compounds such as aldehydes or low molecular weight hydrocarbons, are too large to be only attributed to the fact that some of the tobacco had been substituted with clove material in the cigarette filler. One explanation for these differences is that the tobacco blend used in the tested kretek cigarettes is different from the American-blended filler, notably lower in nitrogen-containing compounds. Accordingly, the specific *in vitro* toxicity is lower, reflecting the lower yields of gas-phase cytotoxic compounds as well as of many TPM mutagenic compounds. More information was gained from mechanistic investigations detailed in the Parts 4 through 6 of the present publication series (Roemer et al., 2014a,b,d) that shed light on other causes for the observed differences between kretek cigarettes and 2R4F in smoke composition and their associated impact on *in vitro* toxicity.

The present results allowed insights into the comparative toxicity of kretek cigarettes, with the aim of providing a basis for hazard characterization. They do not support an increased toxicity of clove-containing cigarettes at the product level.

Conflict of interest

All authors are or were Philip Morris International (PMI) R&D employees. The work reported here was funded by PMI R&D.

Acknowledgments

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Appendix A.

Table A
MS yields per cigarette International Organization for Standardization machine smoking regimen; mean, standard error (S.E.) and number of determinations (*N*).

Parameter (unit)[a]	Sampoerna		Garam		Kretek-R		2R4F	
	Mean	S.E. (*N*)	Mean	S.E. (*N*)	Mean	S.E. (*N*)	Mean	S.E. (*N*)
ISO parameters								
TPM	16.1	0.1 (4)	46.4	0.8 (4)	33.6	0.2 (4)	10.83	0.06 (4)
Tar	14.4	0.2 (4)	39.9	0.7 (4)	29.0	0.1 (4)	8.93	0.10 (4)
Nicotine	0.74	0.04 (4)	1.78	0.02 (4)	1.72	0.02 (4)	0.771	0.002 (4)
Water	0.928	0.029 (4)	4.81	0.17 (4)	2.86	0.05 (4)	1.126	0.031 (4)
Carbon monoxide	8.98	0.34 (4)	17.8	0.8 (6)	18.7	0.2 (4)	12.0	0.1 (4)
Aliphatic dienes								
1,3-Butadiene (μg/cig)	25.4	3.0 (4)	53.2	2.4 (6)	83.5	2.4 (5)	27.6	2.5 (4)
Isoprene (μg/cig)	185	12 (4)	357	29 (6)	518	11 (5)	331	12 (4)
Aldehydes								
Formaldehyde (μg/cig)	38.8	2.4 (5)	68.4	5.5 (10)	60.6	0.7 (10)	18.8	1.4 (5)
Acetaldehyde (μg/cig)	411	21 (5)	670	49 (10)	735	13 (10)	655	17 (5)
Acrolein (μg/cig)	57.5	4.8 (5)	81.1	5.7 (10)	96.7	1.6 (10)	73.9	4.2 (5)
Propionaldehyde (μg/cig)	40.1	2.6 (5)	77.9	4.8 (10)	68.6	1.1 (10)	55.5	2.0 (5)
Aliphatic nitrogen compounds								
Acrylonitrile (μg/cig)	11.3	0.9 (4)	24	1.1 (6)	49.1	0.7 (5)	16.0	0.9 (4)
2-Nitropropane (ng/cig)	5.87	0.74 (4)	10.8	1.3 (4)	19.4	0.4 (5)	17.8	0.3 (4)
Acetamide (μg/cig)	5.05	0.35 (4)	17.6	0.4 (6)	9.22	0.11 (5)	4.90	0.23 (4)

(*continued on next page*)

Table A (*continued*)

Parameter (unit)[a]	Sampoerna		Garam		Kretek-R		2R4F	
	Mean	S.E. (*N*)	Mean	S.E. (*N*)	Mean	S.E. (*N*)	Mean	S.E. (*N*)
Aromatic amines								
2-Naphthylamine (ng/cig)	4.06	0.41 (6)	10.9	1.0 (10)	12.5	0.2 (10)	6.20	0.40 (5)
4-Aminobiphenyl (ng/cig)	0.668	0.062 (6)	1.93	0.17 (10)	2.02	0.03 (10)	1.19	0.10 (5)
o-Anisidine (ng/cig)	1.63	0.10 (6)	3.81	0.34 (10)	3.20	0.05 (10)	2.16	0.06 (5)
o-Toluidine (ng/cig)	47.8	2.8 (6)	117	8 (10)	111	1 (10)	54.0	1.3 (5)
Halogen compounds								
Vinylchloride (ng/cig)	29.7	3.3 (4)	81.8	3.1 (6)	81.3	2.8 (5)	40.9	2.9 (4)
Inorganic compounds								
Hydrogen cyanide (μg/cig)	104	6 (4)	299	12 (8)	162	5 (4)	135	6 (4)
Nitrogen oxides (μg/cig)	97.0	3.4 (4)	189	6 (6)	157	3 (10)	294	14 (4)
Monocyclic aromatic compounds								
Benzene (μg/cig)	39.4	2.7 (4)	86.2	2.7 (6)	84.7	1.2 (5)	49.0	1.4 (4)
Toluene (μg/cig)	58.7	4.5 (4)	138	4 (6)	126	1 (5)	88.9	4.1 (4)
Styrene (μg/cig)	5.40	0.41 (4)	15.3	0.5 (6)	12.4	0.1 (5)	6.53	0.44 (4)
Volatile N-nitrosamines								
NDMA (ng/cig)	<5	ND (4)	<5	ND (4)	<10.0	ND (4)	<5	ND (4)
NMEA (ng/cig)	<10	ND (4)	<10	ND (4)	<20.0	ND (4)	<10	ND (4)
NDEA (ng/cig)	<7	ND (4)	<7	ND (4)	<14.0	ND (4)	<7	ND (4)
NPRA (ng/cig)	<11	ND (4)	<11	ND (4)	<22.0	ND (4)	<11	ND (4)
NBUA (ng/cig)	<9	ND (4)	<9	ND (4)	<18.0	ND (4)	<9	ND (4)
NPY (ng/cig)	<7	ND (4)	<7	ND (4)	<14.0	ND (4)	<7	ND (4)
NPI (ng/cig)	<8	ND (4)	<8	ND (4)	<16.0	ND (4)	<8	ND (4)
Tobacco-specific N-nitrosamines								
NNN (ng/cig)	24.3	2.9 (4)	50.0	5.7 (4)	33.9	1.2 (9)	148	10 (4)
NNK (ng/cig)	21.9	6.0 (4)	23.1	2.5 (4)	20.0	0.8 (10)	129	7 (4)
Phenols								
Phenol (μg/cig)	27.1	1.1 (4)	72.2	2.4 (6)	43.8	0.6 (5)	8.21	0.12 (4)
Catechol (μg/cig)	69.5	2.9 (4)	176	8 (6)	128	2 (5)	45.6	2.1 (4)
Hydroquinone (μg/cig)	61.1	6.0 (4)	126	8 (6)	77.0	1.3 (5)	49.2	5.2 (4)
Eugenol (mg/cig)	2.52	0.07 (4)	8.28	0.19 (6)	4.79	0.11 (5)		
Polycyclic aromatic hydrocarbons								
Benz[*a*]anthracene (ng/cig)	16.6	0.5 (4)	49.0	3.5 (6)	48.8	0.5 (5)	10.9	0.7 (4)
Benzo[*b*]fluoranthene (ng/cig)	9.07	0.51 (4)	22.2	0.5 (6)	19.7	0.3 (5)	6.00	0.19 (4)
Benzo[*j*]fluoranthene (ng/cig)	5.32	0.13 (4)	13.9	ND (6)	11	0.2 (5)	<2	0.09 (4)
Benzo[*k*]fluoranthene (ng/cig)	2.92	0.23 (4)	<8	0.5 (6)	6.25	0.11 (5)	3.60	0.04 (4)
Benzo[*a*]pyrene (ng/cig)	10.6	0.5 (4)	28.3	0.5 (6)	26.3	0.3 (5)	7.71	0.07 (4)
Dibenz[*a*,*h*]anthracene (ng/cig)	<1.0	ND (4)	<1.0	ND (4)	<2.43	ND (5)	<1.0	ND (4)
Dibenzo[*a*,*e*]pyrene (ng/cig)	<0.6	ND (4)	<0.6	ND (4)	NA[b]	ND (5)	<0.6	ND (4)
Dibenzo[*a*,*h*]pyrene (ng/cig)	<0.6	ND (4)	<0.6	ND (4)	0.822	0.015 (5)	<0.6	ND (4)
Dibenzo[*a*,*i*]pyrene (ng/cig)	<1.0	ND (4)	<1.0	ND (4)	<0.550	ND (5)	<1.0	ND (4)
Dibenzo[a,l]pyrene (ng/cig)	<0.6	ND (4)	<0.6	ND (4)	<0.475	ND (5)	<0.6	ND (4)
Indeno[1,2,3-*cd*]pyrene (ng/cig)	4.73	ND (4)	9.85	0.70 (6)	10.1	0.1 (5)	3.44	0.11 (4)
5-Methylchrysene (ng/cig)	<7	ND (4)	<23	ND (4)	<1.00	ND (5)	<0.4	ND (4)
Elements								
Arsenic (ng/cig)	<1.7	ND (3)	<5	ND (3)	5.83	0.22 (4)	2.67	0.13 (3)
Cadmium (ng/cig)	8.79	0.19 (3)	48.1	2.9 (3)	111	3 (4)	30.4	1.5 (3)
Chromium (ng/cig)	<1.7	ND (3)	<5	ND (3)	6.41	ND (3)	<1.0	ND (3)
Nickel (ng/cig)	<3.3	ND (3)	<10	ND (3)	<6.00	ND (3)	<2.0	ND (3)
Lead (ng/cig)	8.06	0.49 (3)	30.7	0.49 (3)	24.5	0.8 (4)	10.5	0.2 (3)

ND, no standard error calculated as some determinations were below quantification limit.
[a] *Abbreviations:* NNN: N′-nitrosonornicotine; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NDMA: N-nitrosodimethyl amine; NMEA: N-nitrosomethylethylamine; NDEA: N-nitrosodiethylamine; NPRA: N-nitrosodi-n-propylamine; NBUA: N-nitroso di-n-butylamine; NPY: N-nitrosopyrrolidine; NPI: N-nitrosopiperidine; Garam, *Gudang Garam International Filter*; Sampoerna, *A Mild 16*; Kretek-R, *kretek reference cigarette*.
[b] Not analyzed.

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